

Foliar flavonoids of the genus *Hedysarum* and related genera (tribe Hedysareae-Leguminosae)

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• 뭇 황기속과 근연속(콩科)에서의 잎의 Flavonoids에 관한 연구

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Abstract

Five flavonoid aglycones and the xanthone mangiferin were identified from hydrolysed leaf extracts of sixteen species of *Hedysarum* and its related four genera (tribe Hedysareae *sensu stricto*). The flavonol quercetin was found in all taxa and kaempferol was present in all except two species. Myricetin was characterised in only six species without any apparent correlation with any of the taxonomic treatments of the tribe. The presence of isorhamnetin in eight species showed some correlation with habitat (xeric conditions), but mangiferin was found only in species of the subsection Obscura. The flavonoid profiles of tribe Hedysareae have not proved useful in the delimitation of generic boundaries.

Introduction

The genus *Hedysarum* includes about 100 species. Fedtschenko (1899, 1902; Table 3) first proposed that *Hedysarum* should be divided into two subgenera, including two sections and five subsections. More recently Polhill (1981) suggested that the tribe Hedysareae *sensu stricto* comprised seven genera: *Hedysarum*, *Taverniera*, *Onobrychis*, *Ebenus*, *Eversmannia*, *Stracheya* and

Sartoria. Following Bentham (1865), Taubert (1894) and Hutchinson (1964), Ohashi (1971) included *Alhagi* in the tribe. However, the delimitation of the genera on morphological grounds is very difficult, and the classification of the infrageneric groups of *Hedysarum* is still somewhat confused (Rollins, 1940 ; Polhill, 1981 ; Thulin, 1985 ; Choi, 1988). Harborne (1971) analyzed nine *Hedysarum* species and representatives of other genera in the Hedysareae for their flavonoid constituents. He found that the xanthone, mangiferin, was a potentially useful taxonomic marker in the group. Therefore, in this study, the flavonoid survey of nine *Hedysarum* species and six species of four related genera was undertaken to conform or refute his findings.

Materials and Methods

Several individuals, comprising fifteen species, were surveyed from each of the following genera ; *Hedysarum*, *Taverniera*, *Alhagi*, *Onobrychis*, and *Ebenus*. Leaf fragments of herbarium specimens from the Royal Botanic Gardens, Kew (K), British Museum, Natural History (BM), and Department of Biology, Inha University (IUI), and living collections at Kew Gardens were used for flavonoids analysis (Table 1).

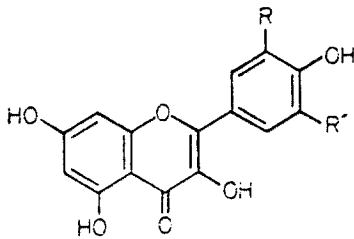
80% methanolic leaf extracts of each species were acid hydrolysed with 2N HCl at 100°C for 40 minutes. The hydrolytes were cooled and extracted with ethyl acetate. The concentrated ethyl acetate fractions were taken into 95% ethanol and chromatographed on cellulose TLC plates along with standard markers in BAW (*n*-butanol-acetic acid-water ; 4 : 1 : 5), 50% HOAc (50% aqueous acetic acid), Forestal (acetic acid-conc. HCl-water ; 30 : 3 : 10) and CAW (chloroform-acetic acid-water ; 30 : 15 : 2). The aglycones were visualised under UV light + ammonium vapour (Table 2.)

Results and Discussion

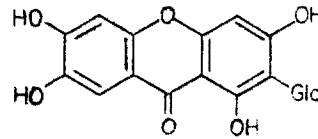
The foliar flavonoid aglycones which have been isolated from members of tribe are tabulated in table 3. A total of five flavonoid aglycones and the xanthone, mangiferin, were identified in the examined taxa.

The flavonoid, quercetin (1) was present in all species surveyed, and kaempferol (2) was found in all taxa except *Hedysarum coronarium* and *H. vicioides* var. *japonicum*. On the other hand, myricetin (3) was restricted in its distribution, being found only in subsections Subacaulia, Multicaulia and Membranaceae and genera *Taverniera* and *Ebenus*. The distribution pattern of myricetin was not congruent with any of the traditional taxonomic treatments.

Isorhamnetin (4) appeared as the characteristic flavonoid in those species growing in a xeric habitat. Thus, its occurrence in these plants may be associated with an adaptation to xeric habitat, rather than a reflection of phylogenetic relationships. In fact, the flavonoid profiles obtained for



1. Quercetin ; R=OH, R'=H
2. Kaempferol ; R=R'=H
3. Myricetin ; R=R'=OH
4. Isorhamnetin ; R=OMe, R'=H



5. Mangiferin ;
Glc=Glucose

species in the tribe Hedysareae did not lend any support for current classifications or help in the delimitation of the genera.

On the other hand, mangiferin (5) was found only in *Hedysarum vicioides* var. *japonicum* of subsection Obscura in this survey. Harborne (1971) also reported mangiferin from three other species of subsection Obscura. Morphologically subsection Obscura has been distinguished from other subsections within section Gamotion by the presence of bundle-sheath extensions in lateral leaf veins (Choi, 1988) and the absence of ribs and spines on the fruit surfaces. Therefore, the presence of mangiferin seems to be an important taxonomic marker for the determining the phylogeny and classification within the genus *Hedysarum*.

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摘 要

뫨황기속과 근연속(콩과) 15종의 잎의 flavonoids 성분을 조사한 결과 6개의 flavonoids가 검출되었으며, 이 중 mangiferin은 뫨황기속의 Obscura亞節에서만 검출되었다. Flavonol계통인 quercetin과 kaempferol은 이 식물군에서 가장 일반적으로 발견되었고, myricetin은 계통학적 유연관계와 관계없이 각 분류군에서 불규칙하게 나타났으며, isorhamnetin은 건조지에 자라는 분류군에서 주로 검출되었다. 뫨황기속의 속간 또는 속내 분류군에 있어, flavonoids의 존재여부와 전통적 분류체계 사이에는 뚜렷한 일치점이 발견되지 않았다.

Table 1. The species of the genus *Hedysarum* and its related genera in the tribe Hedysareae examined for the flavonoid composition.

1. <i>Hedysarum coronarium</i> L. : Living collection at Kew Gardens (collecting date unknown).
2. <i>Hedysarum vicioides</i> var. <i>japonica</i> Choi et Ohashi : Japan, Mt. Fubo, 30 June 1986, Choi 369 (IUI).
3. <i>Hedysarum flavescence</i> Rgl. et Schm. ex Fedch. : Living collection at Kew Gardens (collecting date unknown).
4. <i>Hedysarum microphyllum</i> Turcz. : Turkmenia, 29 July 1974, M. Lomomocova & L. Paniluk 2718 (K).
5. <i>Hedysarum nitidum</i> Willd. : Turkey, B9 Erzurum, 24 July 1966, Davis 47304 (K).
6. <i>Hedysarum varium</i> Willd. : Turkey, Ezincan, 1967, M. C. Watson 3244 (K).
7. <i>Hedysarum singarense</i> Boiss. et Haussk. : Iraq, Mam, 17 June 1970, S. Omar 37732 (K).
8. <i>Hedysarum membranaceum</i> Coss. et Bal. : Moroc, Arni, 20 Aug 1990, Jos Lewalle 13119 (BM).
9. <i>Hedysarum fruticosum</i> Pall. : Mongolia, Ulan-Ude, 6 July 1952, M. Popov 3842 (K).
10. <i>Taverniera breviata</i> Thul. : Omam, Dhofar, Mirbat, 19 Sept. 1984, A. G. Miller 6175 (K)
11. <i>Taverniera multoidea</i> Thul. : N. E. Somalia, 6/1/84, C. Barbier 1021 (K).
12. <i>Alhagi maurorum</i> Medik. (1) : Iran, Mazandaran, 6 June 77, T. F. Hewer 3918 (K).
13. <i>Alhagi maurorum</i> Medik. (2) : Afghanistan, Kabul, 4 June 1971, C. Grey-Wilson & T. F. Hewer 1019 (K).
14. <i>Ebenus lagunoides</i> Boiss. : Turkey, B.6 Malayta, 11 Aug. 1967, A. R. Mitchell, A. C. & W. 3546 (K).
15. <i>Onobrychis arenaria</i> DC. : Living collection at Kew Gardens (Collected from Armenia).
16. <i>Onobrychis ptolemaica</i> DC. : Saudi Arabia, South of Tayma, 12/3/86, I. S. Collenette 5736 (K).

Table 2. Rf values and colours under UV light with NH₃ of leaf flavonoids.

Compound	Rf × 100				Colour under UV + NH ₃
	BAW	CAW	50%HOAC	FORESTAL	
Quercetin	76	13	23	42	bright yellow
Kaempferol	96	44	38	61	bright yellow
Myricetin	45	6	nd	29	bright yellow
Isorhamnetin	95	70	46	nd	bright yellow
Mangiferin	38	4	51	66	yellow
Unknown	nd	nd	47	nd	yellow

nd = not determined

Table 3. Distribution of leaf flavonoids in species of the genus *Hedysarum* and its related genera in the tribe Hedysareae. Abbreviations: Qu=Quercetin ; Km=Kaempferol ; My=Myricetin ; Qu3'ME=Isorhamnetin ; Mang=Mangiferin ; Unkn=Unidentified flavonoid ; nd=not determined. * from Harborne(1971)

	Qu	Km	My	Qu3'ME	Mang	Unkn
Genus <i>Hedysarum</i>						
Subgenus Hedysarum						
Sect. Hedysarum						
<i>H. coronarium</i>	+	-	-	-	-	-
Sect. Gamotion						
Subsect. Obscura						
* <i>H. hedysaroides</i>	nd	nd	nd	nd	+	nd
* <i>H. viciodes</i>	nd	nd	nd	nd	+	nd
* <i>H. boreale</i>	nd	nd	nd	nd	+	nd
<i>H. vicioides</i> var. <i>japonica</i>	++	-	-	-	++	-
<i>H. flavescence</i>	++	±	-	-	-	-
Subsect. Subacaulia						
<i>H. microphyllum</i>	++	++	+	±	-	-
Subsect. Multicaulia						
<i>H. nititum</i>	++	±	+	-	-	-
<i>H. varium</i>	++	++	±	+	-	-
Subsect. Crinifera						
<i>H. singarense</i>	++	+	-	-	-	-
Subsect. Membranacea						
<i>H. membranaceum</i>	++	+	-	-	-	-
Sugenus Heteroloma						
<i>H. fruticosum</i>	++	++	-	++	-	-
Genus <i>Taverniera</i>						
<i>T. breviata</i>	+	++	±	±	-	-
<i>T. multoida</i>	+	++	-	+	-	-
Genus <i>Alhagi</i>						
<i>A. maurorum</i> (1)	+	++	-	++	-	-
<i>A. maurorum</i> (2)	+	++	-	++	-	-
Genus <i>Ebenus</i>						
<i>E. lagunoides</i>	++	±	+	-	-	+
Genus <i>Onobrychis</i>						
<i>O. arenaria</i>	++	±	-	-	-	-
<i>O. ptolemaica</i>	++	+	-	-	-	-

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